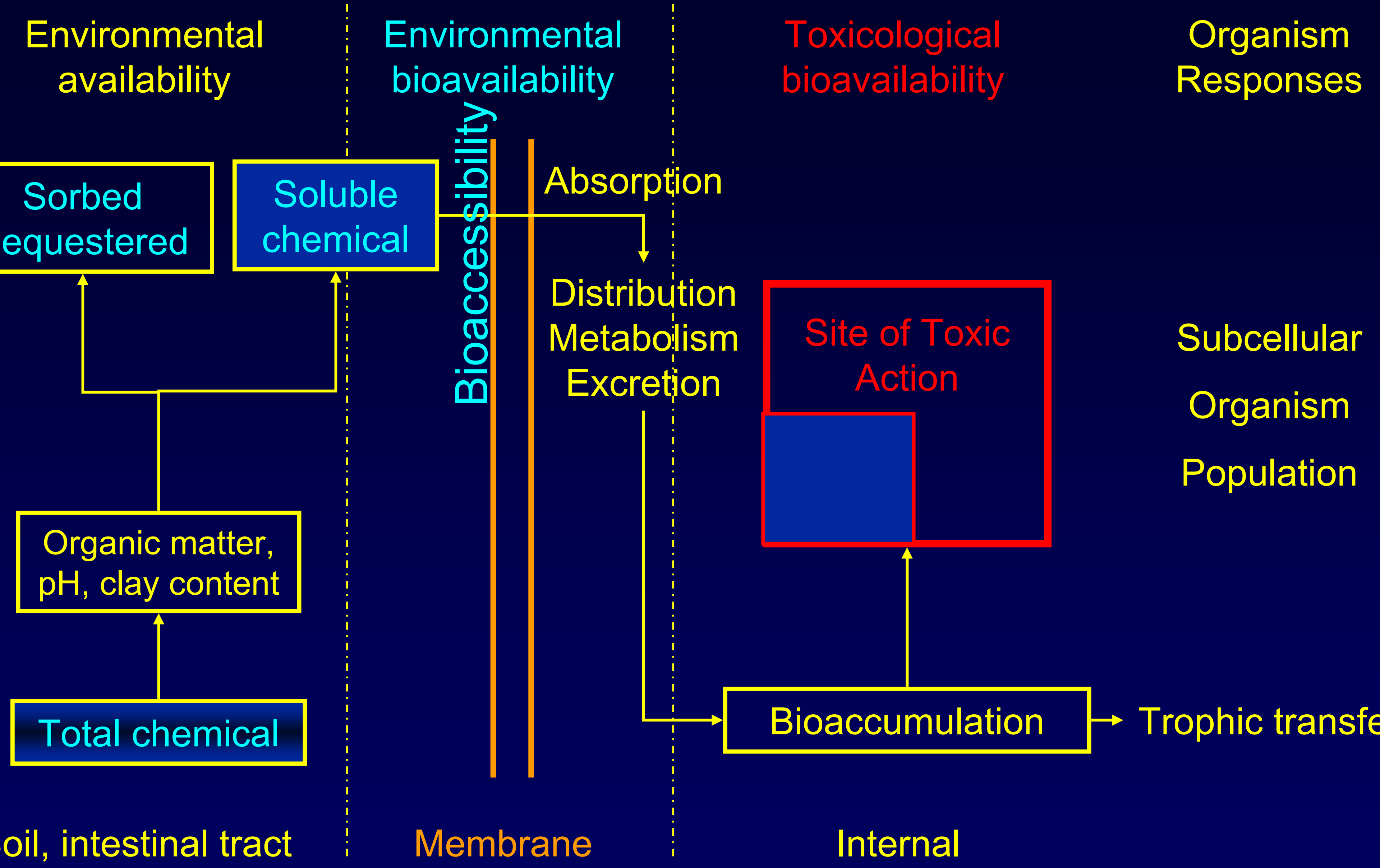
Assessing the bioavailability of PAHs to soil invertebrates: Theory, techniques and applications

R.P. Lanno

Department of Entomology
Ohio State University

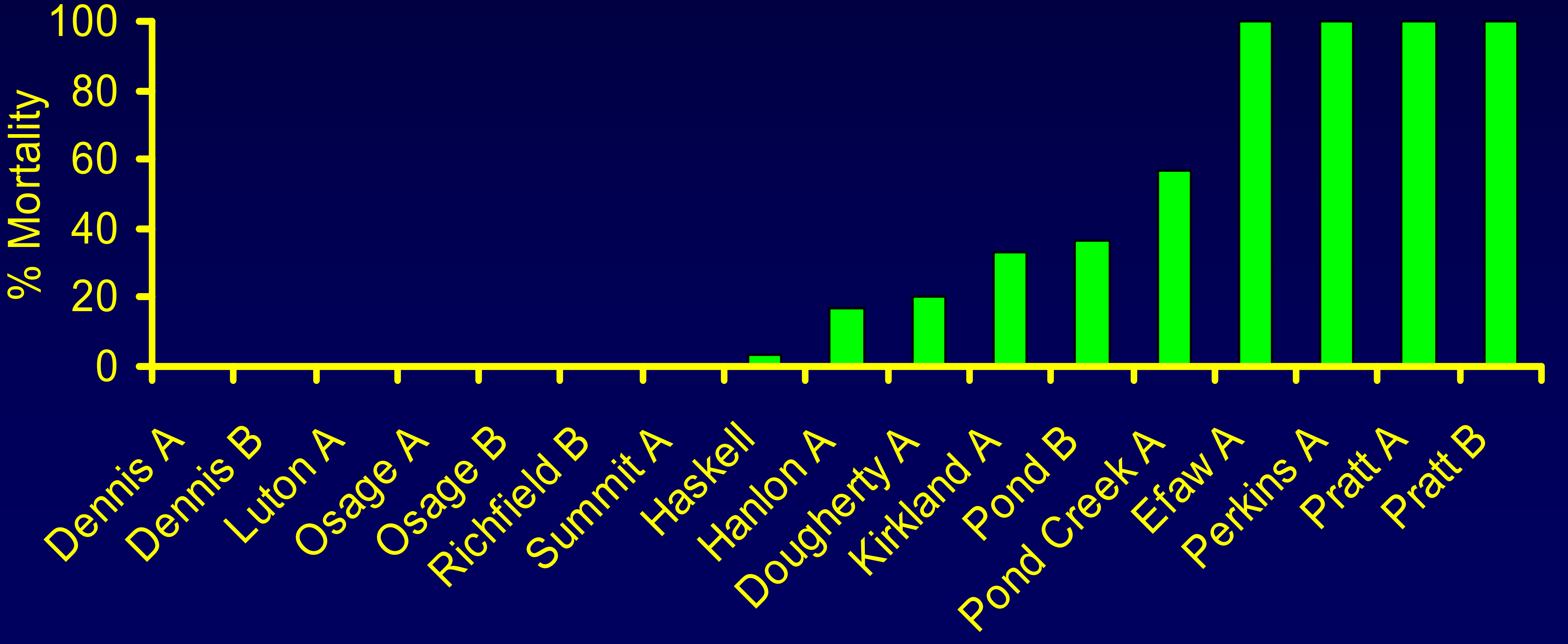
Outline of Presentation

- Bioavailability/bioaccessibility
- Measures of bioavailability
  - Chemical, Biological, Biomimetic
- Uncertainty – accuracy and precision
- Models for predicting bioavailability



Schematic model of bioavailability

Response of earthworms in soils differing in physical/chemical characteristics, BUT all spiked with Pb (2000 mg/kg)



Indirect measures (surrogate)

Direct measure

Chemical measures

~~Total chemical~~

Solvent extractions

Solid-phase extractions

Biomimetic Sampling Devices

Correlation

Calibration

membrane

Biological measures

Toxicity tests

Biomarkers

Bioaccumulation

Critical Body Residue (CBR)

Measures of bioavailability/bioaccessibility

Bioavailability in ecological risk assessment

- Dose-response for risk characterization
- Reduce uncertainty in risk estimation:
  - Precision
  - Accuracy
  - Measured chemical concentrations (how?)

Precision

- OECD Enchytraeid Reproduction Test - coefficient of variation (CV) around the mean number of juveniles is not higher than 50%
- Individual PAH levels in soils before and after supercritical fluid extraction (SFE) - CV – 4-9%
- Usually much greater variability in biological response

Accuracy

Dose

- Analytical chemistry – reference standards, recovery
- Bioavailability – bioassay issues
  1) Toxicological bioavailability - Number of moles of chemical at site of toxic action
     - Receptor binding>tissue residue>extractions of medium>total chemical
  2) Kinetics (chemical, bioassay response)
  3) Assumptions
  - Constant exposure; homogeneity of matrix
- Bioaccessibility
  - What fraction of the chemical does organism encounter?
  - Matrix independence

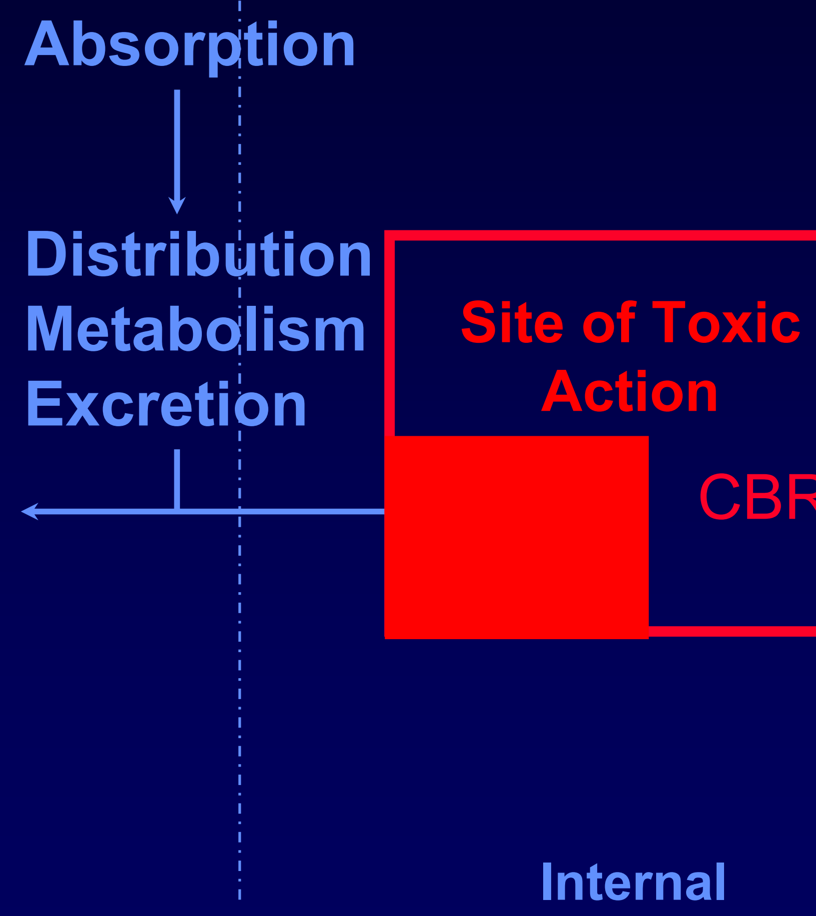
1) Toxicological bioavailability

Critical Body Residue (CBR)

- residue of chemical in an organism associated with a biological response (e.g., lethality

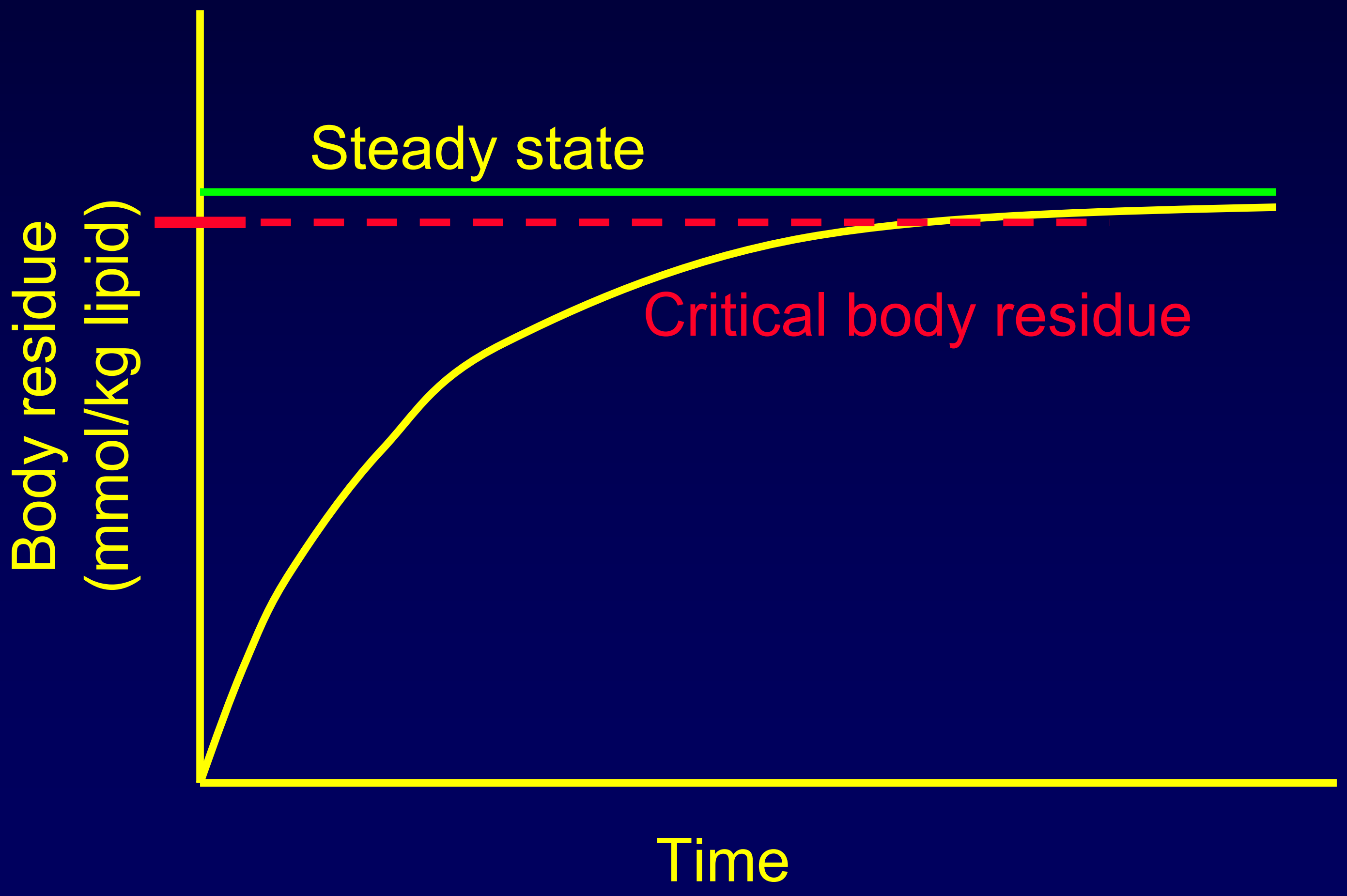
CBR Hypothesis

- for a given chemical with a specific mode of toxic action (e.g., PAHs), CBRs for equally sensitive organisms will be the same (e.g., 2-8 mmol/kg)



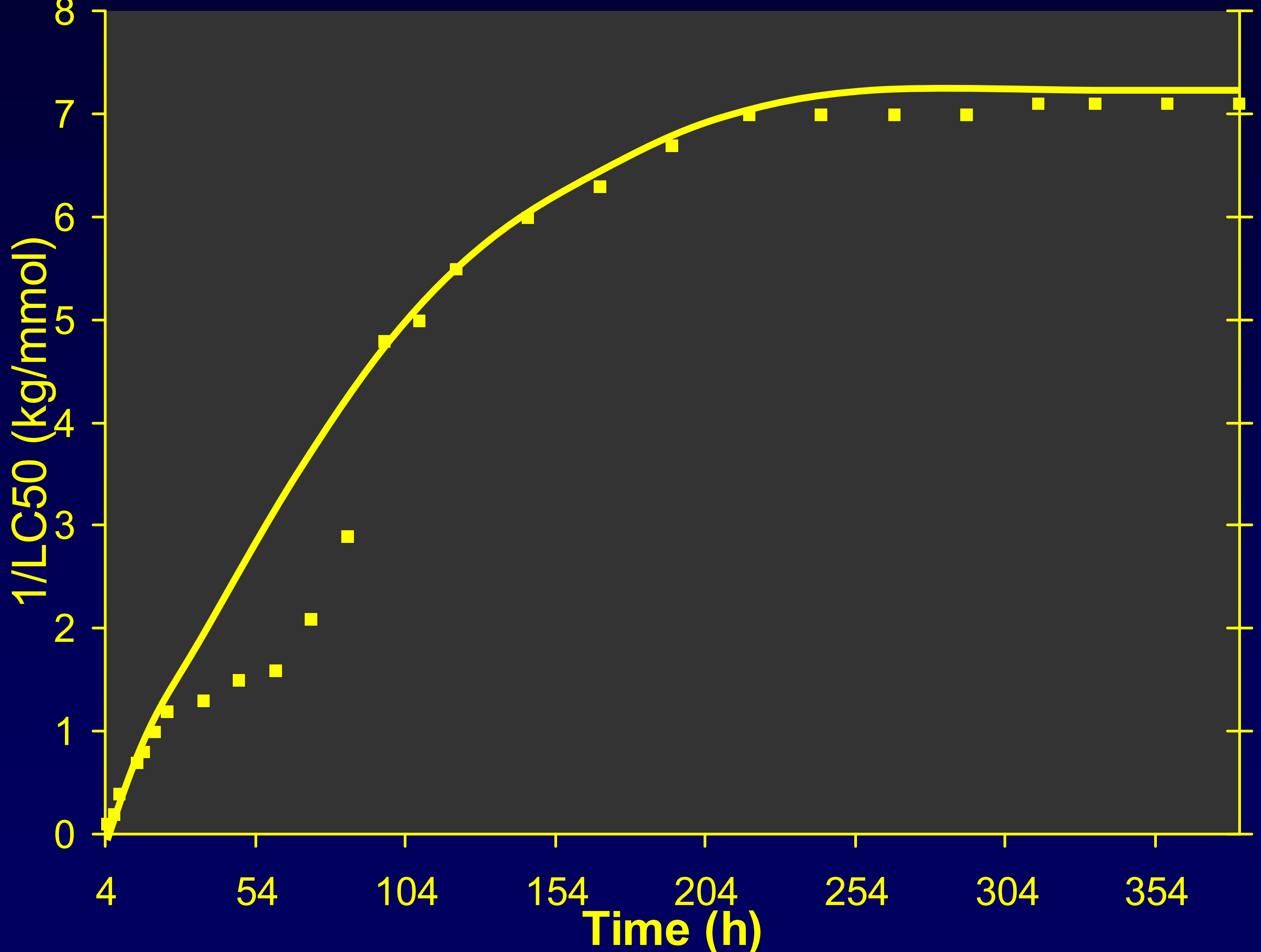
Kinetics - 1CFOK model

$$C_{org}(t) = C_{org}(0)e^{-k_2 t} + \frac{k_1 C_{medium}}{k_2}(1 - e^{-k_2 t})$$



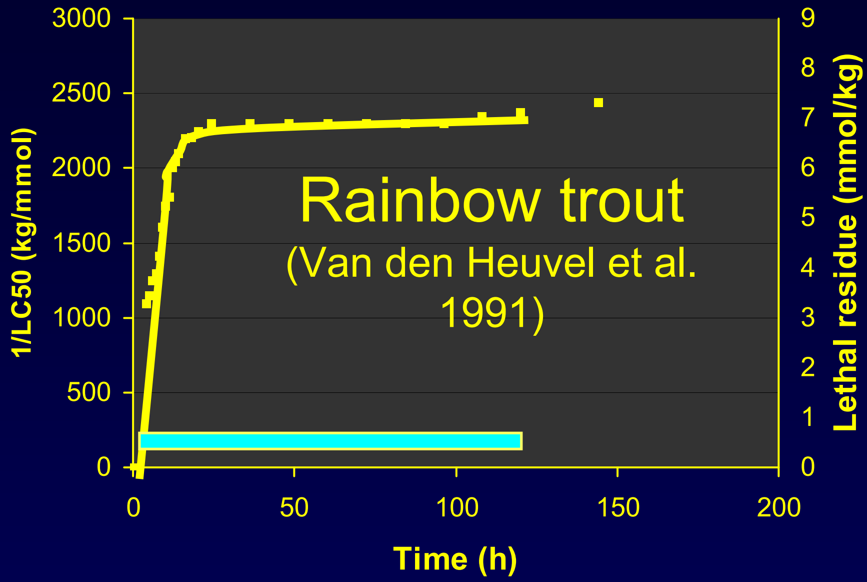
Kinetics – Toxicity curve

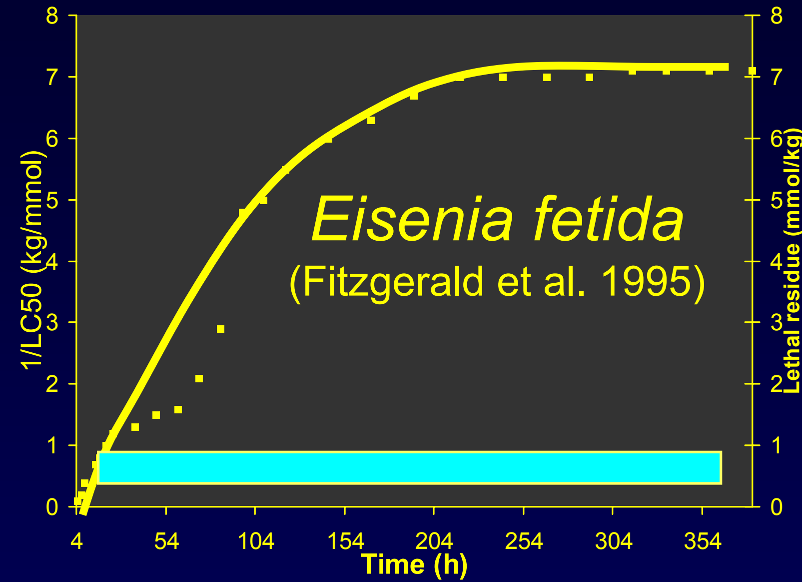
E. fetida – exposed to PCP

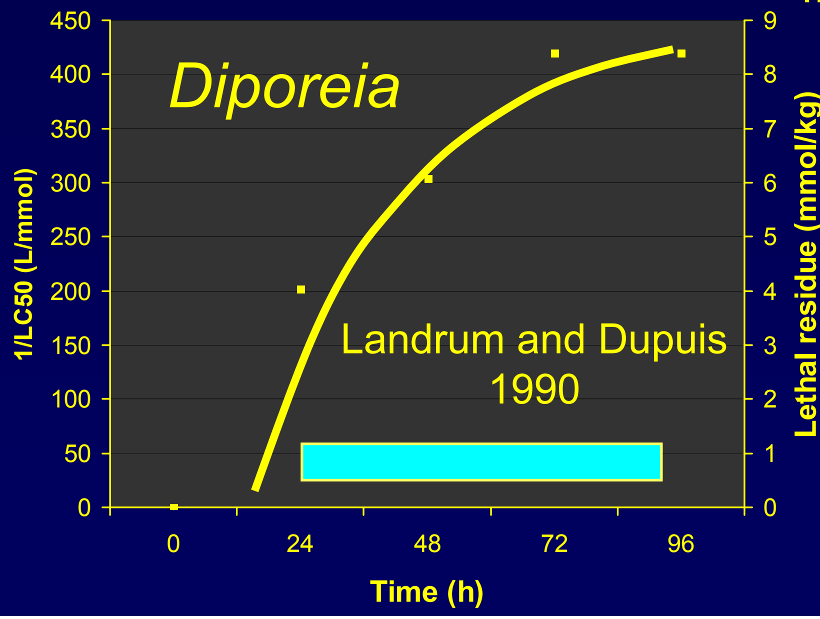


Comparison of lethal CBRs for Pentachlorophenol

Lanno and McCarty 1997







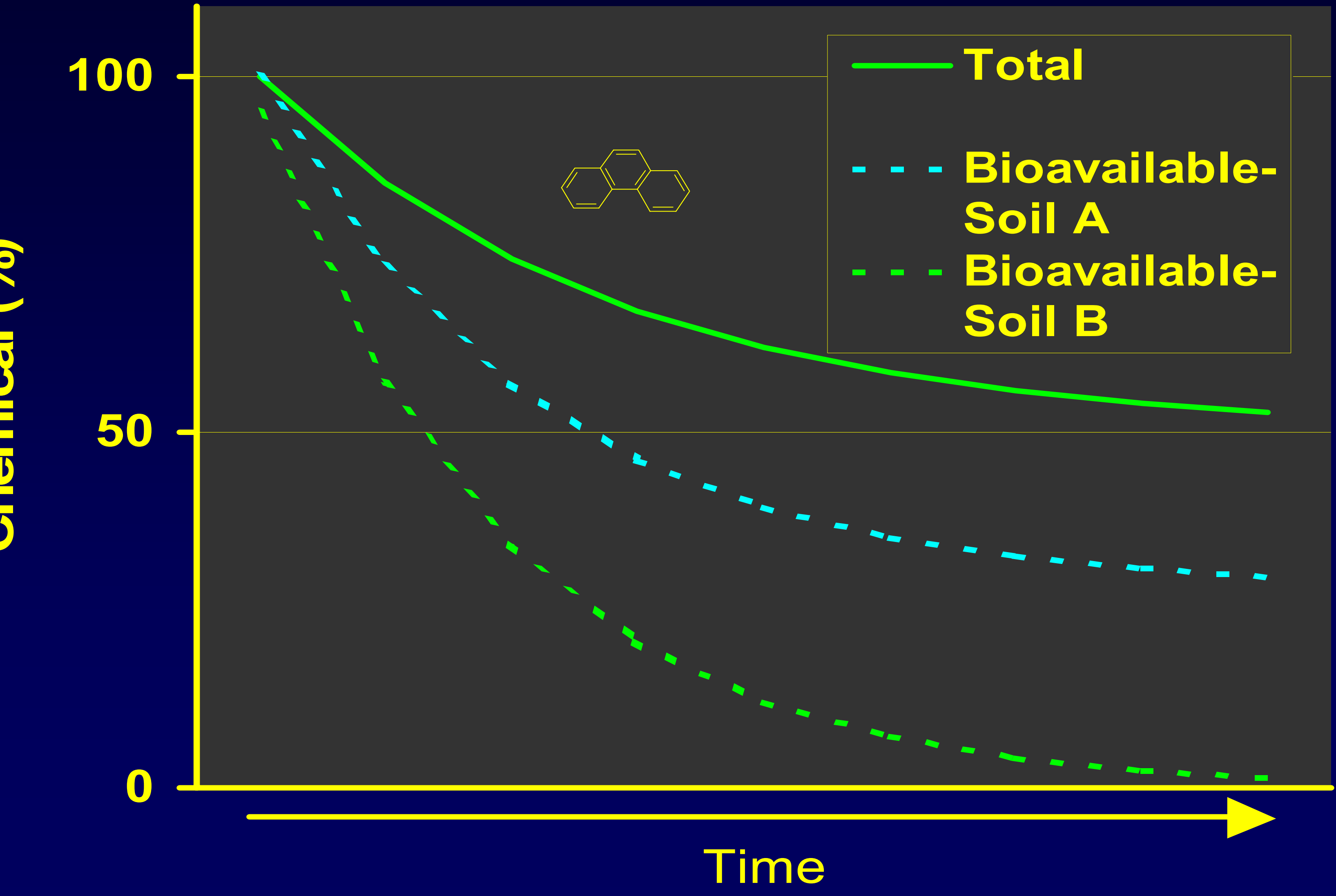
<u>ILL range</u>
0.004-0.16
(418X)

Environmental availability

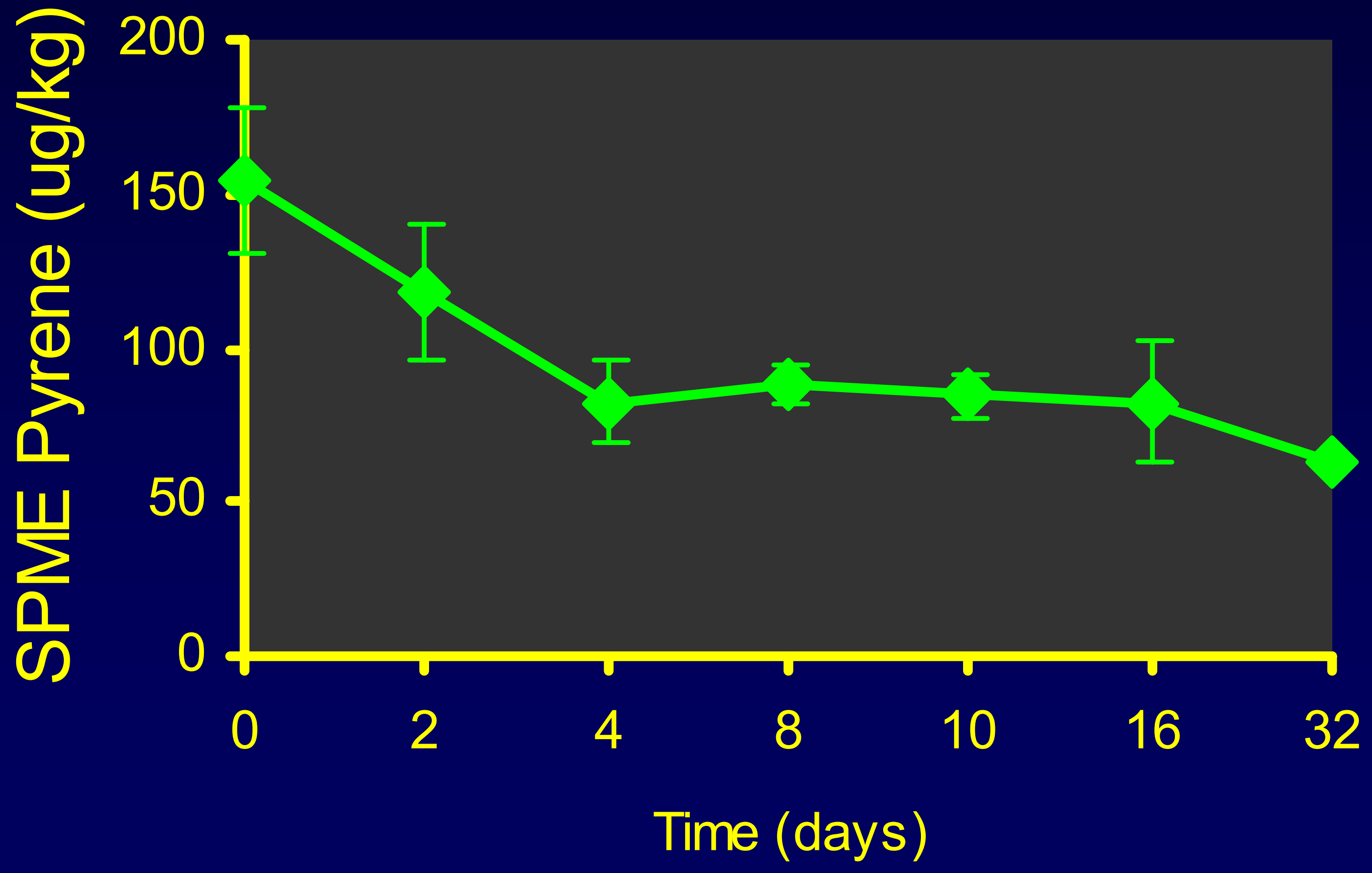
<u>CBR range</u>
0.1-0.7
(7X)

Toxicological bioavailability

Partitioning kinetics of organic chemicals in soil over time



Potentially bioavailable pyrene amended in soil



Summary - Dose accuracy

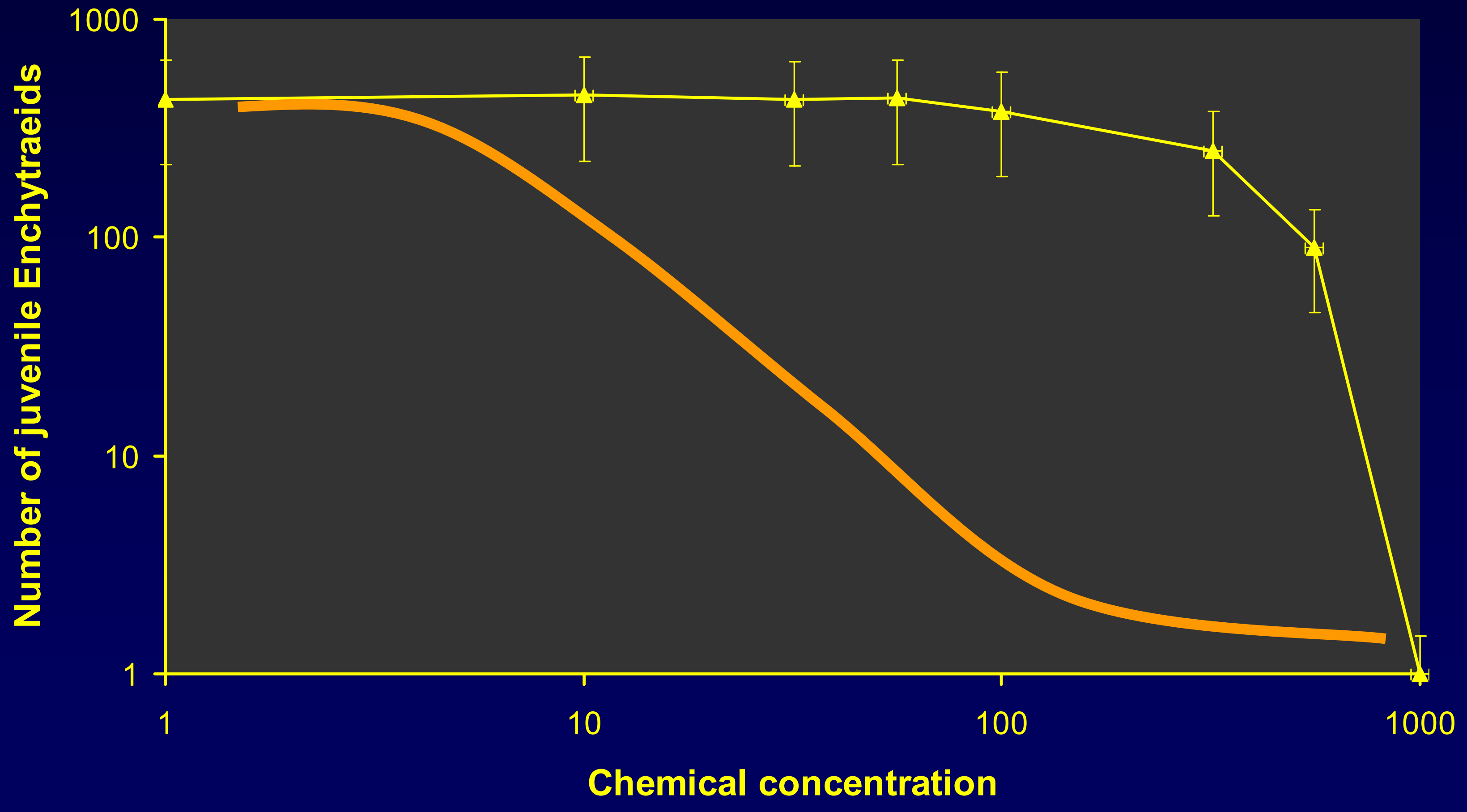
Bioassay response – measured at steady state:

- Chemical uptake kinetics of organisms (e.g., 1CFOK)
- Toxicokinetics for organism response (e.g., toxicity curves)
- Partitioning kinetics – assumption of constant exposure concentration

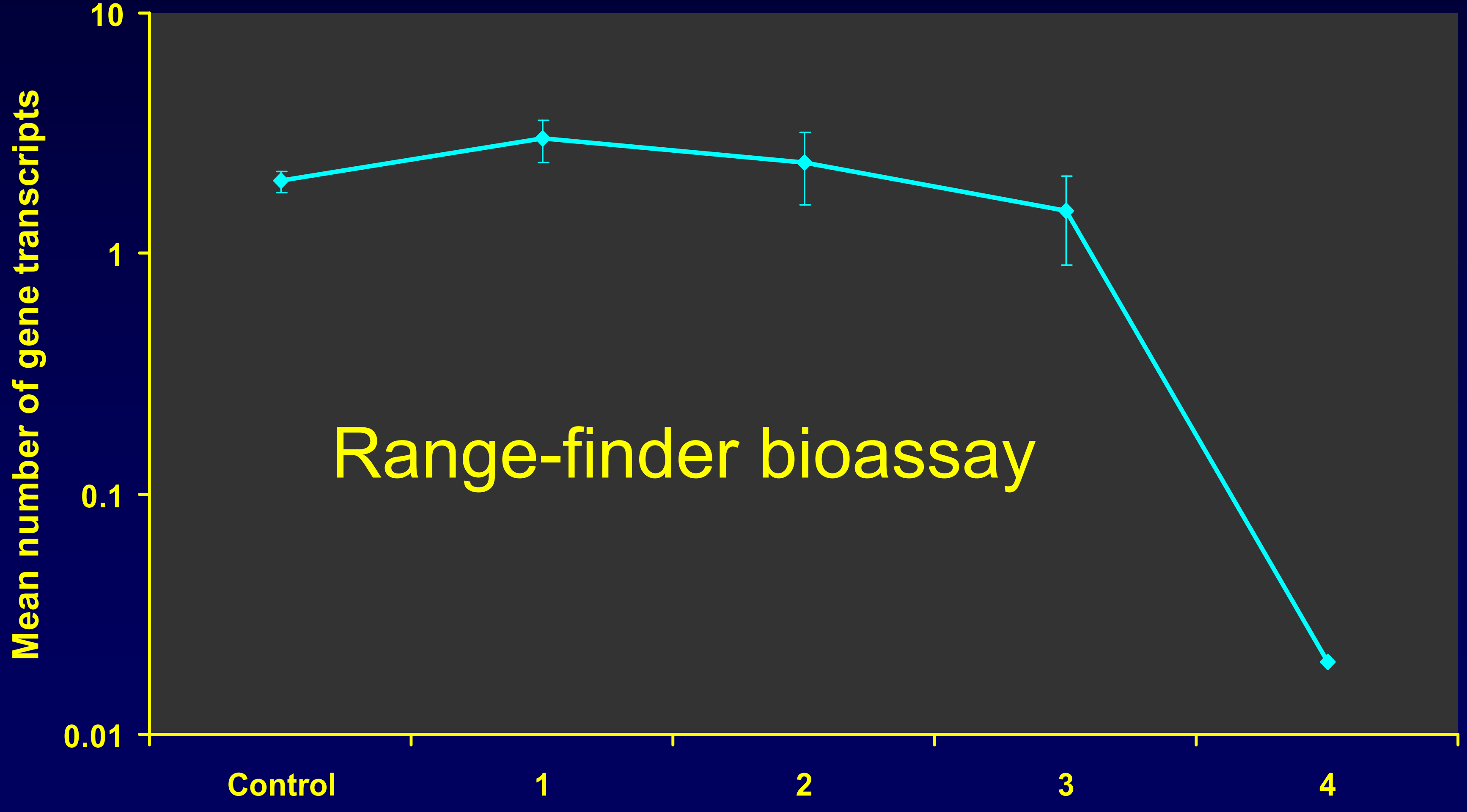
Accuracy - Bioassay response

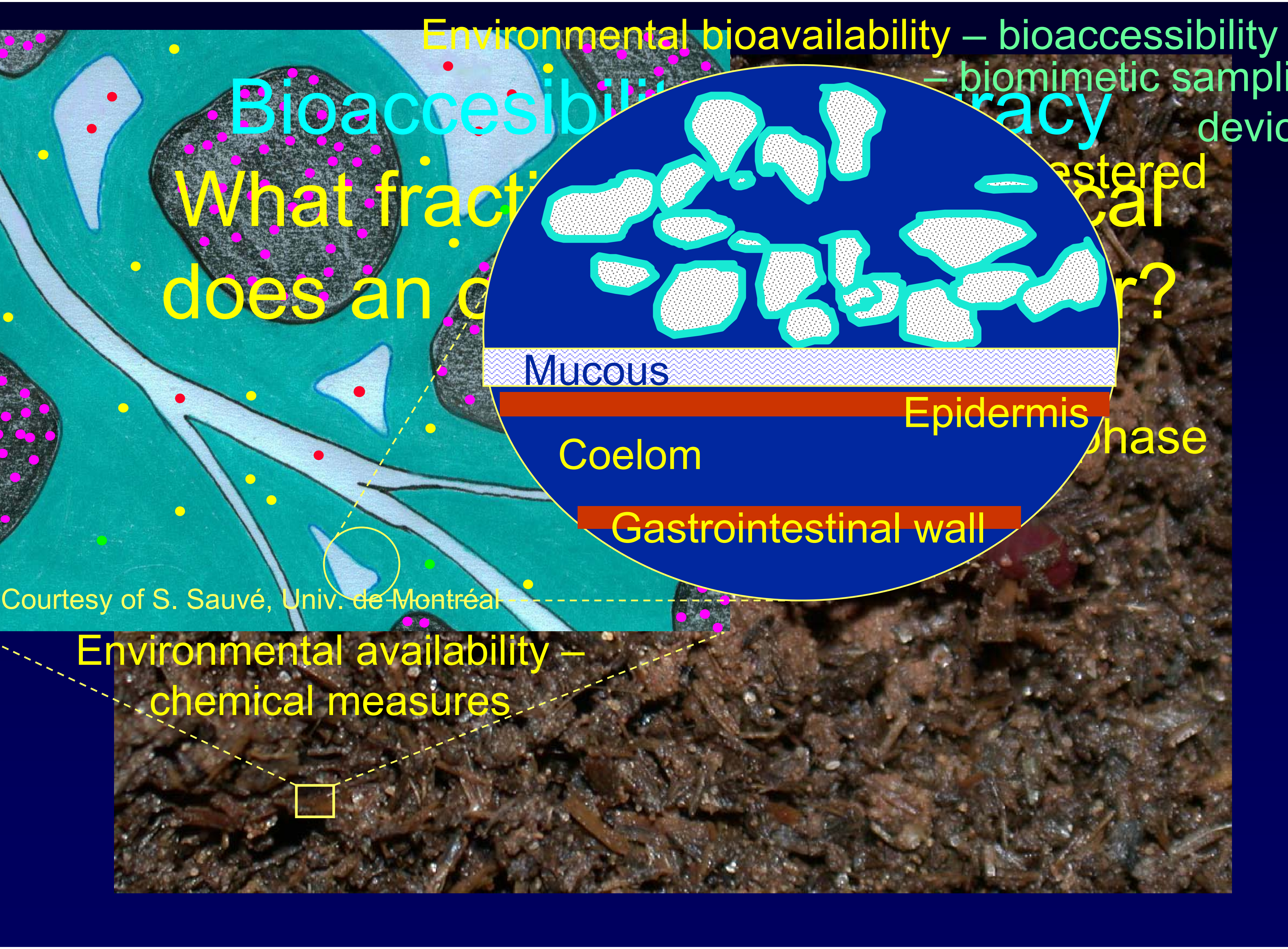
- ??? – We don’t know what the true value of a bioassay response should be
- Reference toxicant tests – control chart
- Adult synchronicity
- Shape of the dose-response curve

All-too-often-seen dose-response



Example from the literature

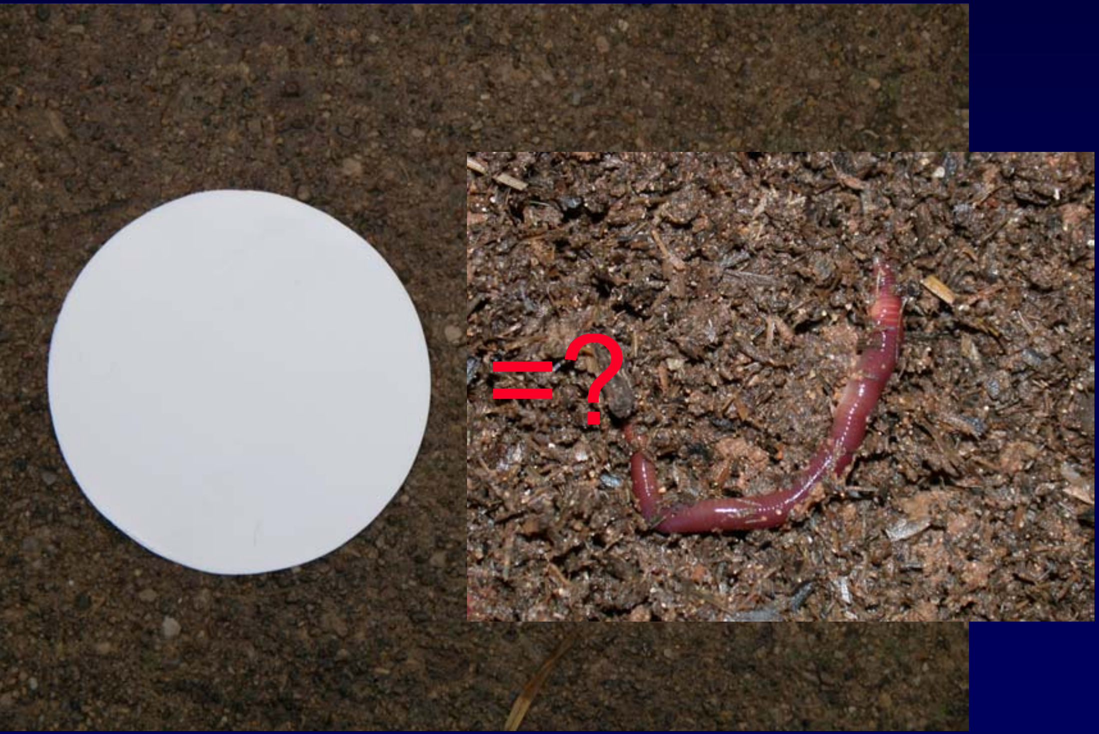



Environmental bioavailability – bioaccessibility
Mucous
Epidermis
Coelom
Gastrointestinal wall
Courtesy of S. Sauvé, Univ. de Montréal
Environmental availability –
chemical measures

Solvent extraction may offer a good measure of environmentally bioavailable chemical

PAH-type	Initial	Post SFE extraction	Mass extracted	Percent removed
		ug/g soil		**%**
2-Ring	746	26	720	97
3-Ring	1,350	56	1,294	96
4-Ring	979	171	808	83
5-Ring	414	232	182	44
6-Ring	189	164	25	13
Total	3,680	649	3,030	82
Soil invertebrate survival (%)				
E. fetida	0	100		
E. albidus	0	90		

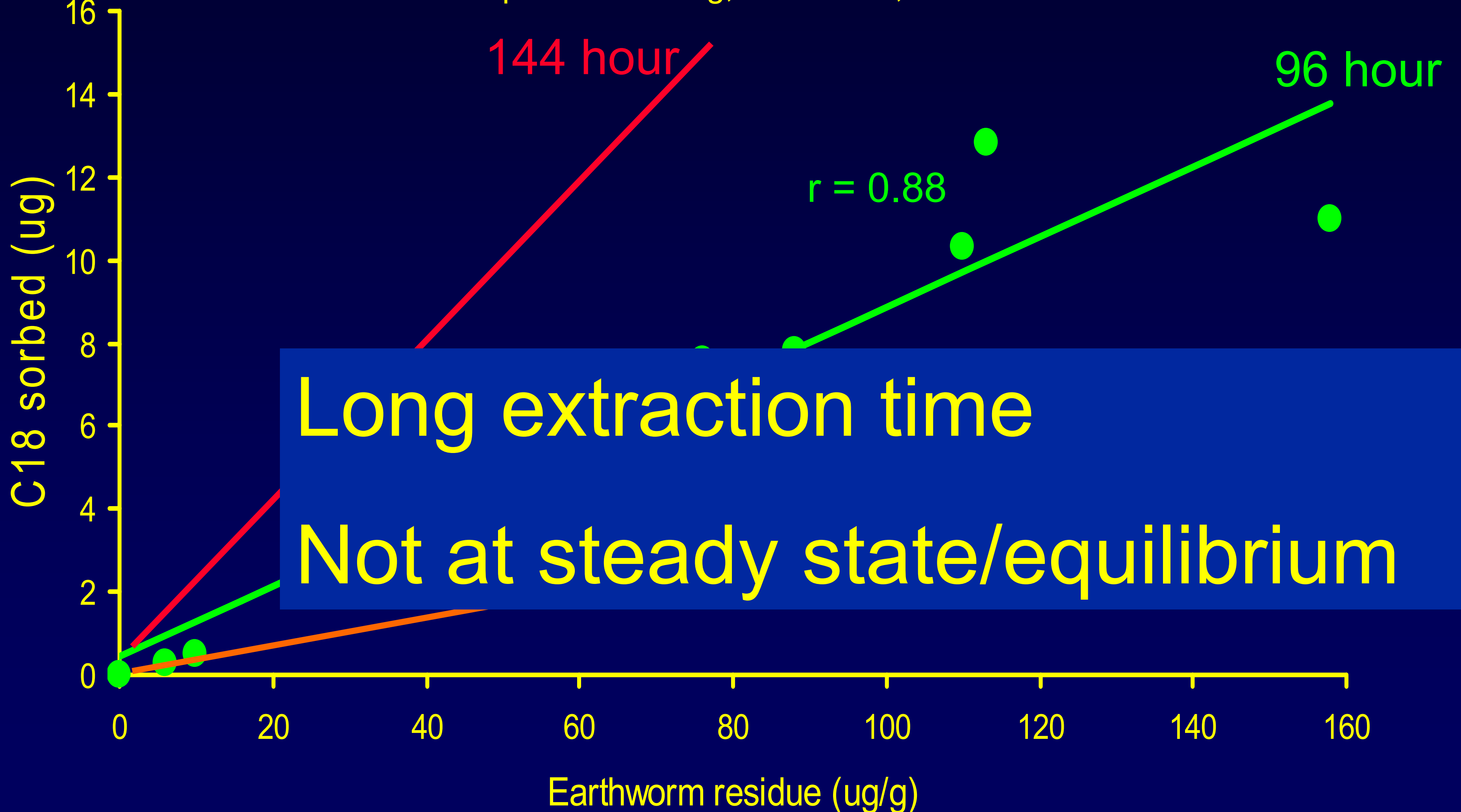
C18 or Empore™ Disks



- Solid-phase extraction disk
- C18 (octadecyl) particles entrapped in by Teflon™

Relationship between C18 disk sorption of total DDT and earthworm residues

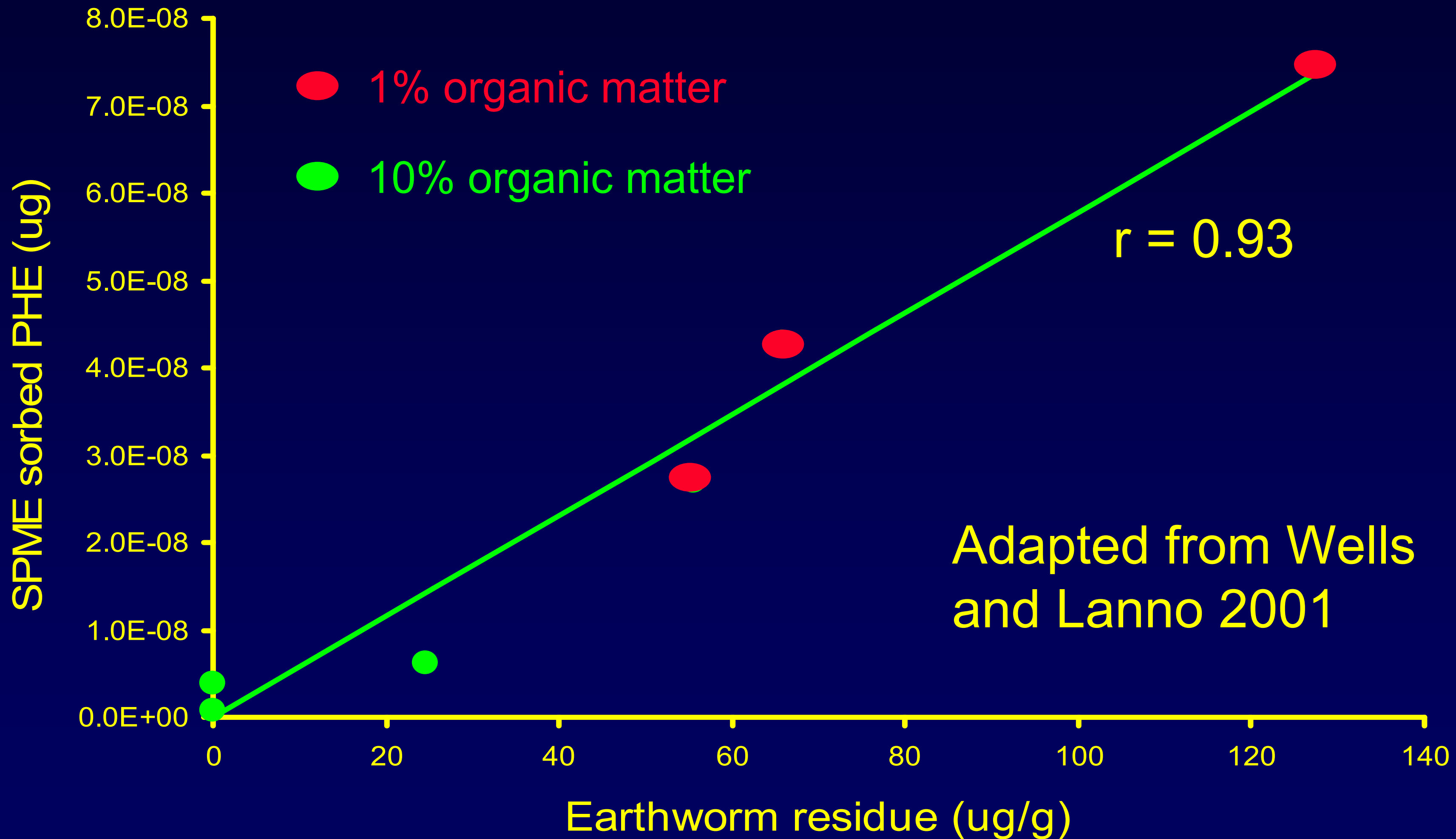
Adapted from Tang, Robertson, & Alexander 1999



Long extraction time

Not at steady state/equilibrium

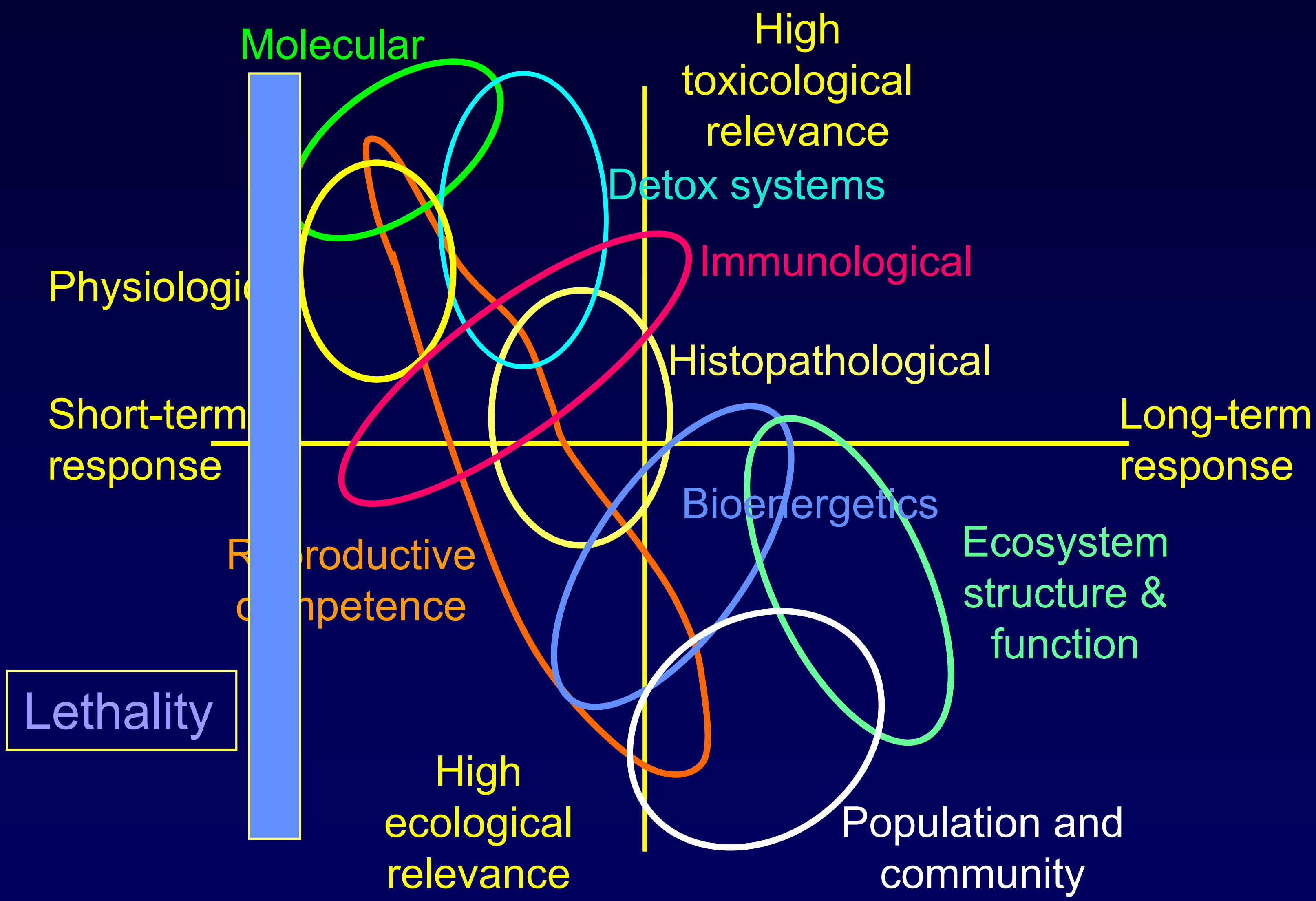
Relationship between SPME sorption of PHE and earthworm PHE residues

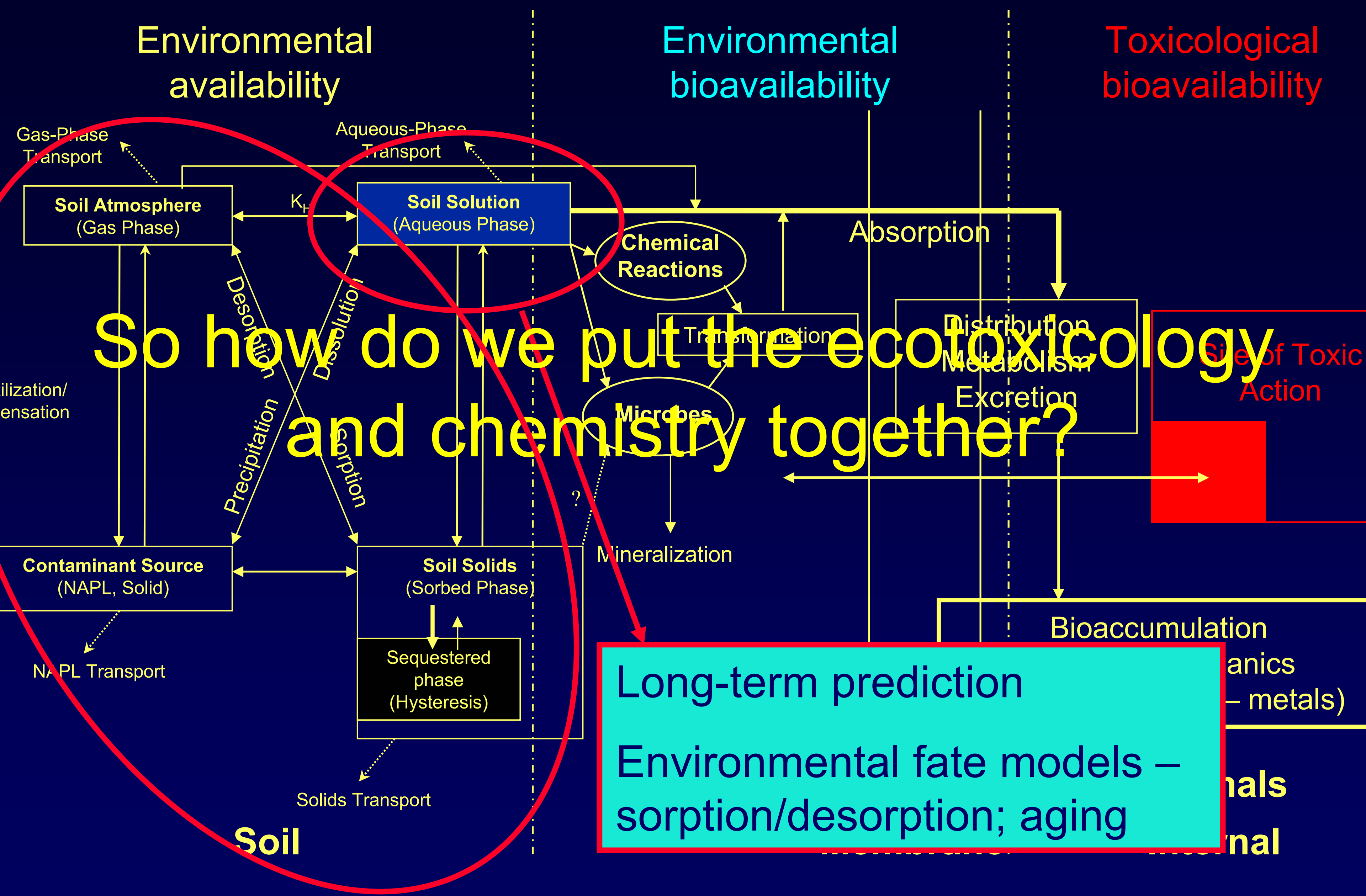


What can we change?

- Bioassay design
  - Change method of exposure
  - Change method of measuring exposure
- Design experiments including analytical chemistry, ecotoxicology, physiology, biochemistry, and environmental chemistry, expertise to increase precision and accuracy of dose-response
- Develop integrated models of dose-response
- Develop tests with new species

Ecological relevance - Endpoints in biological response (Adapted from Adams et al.1989)



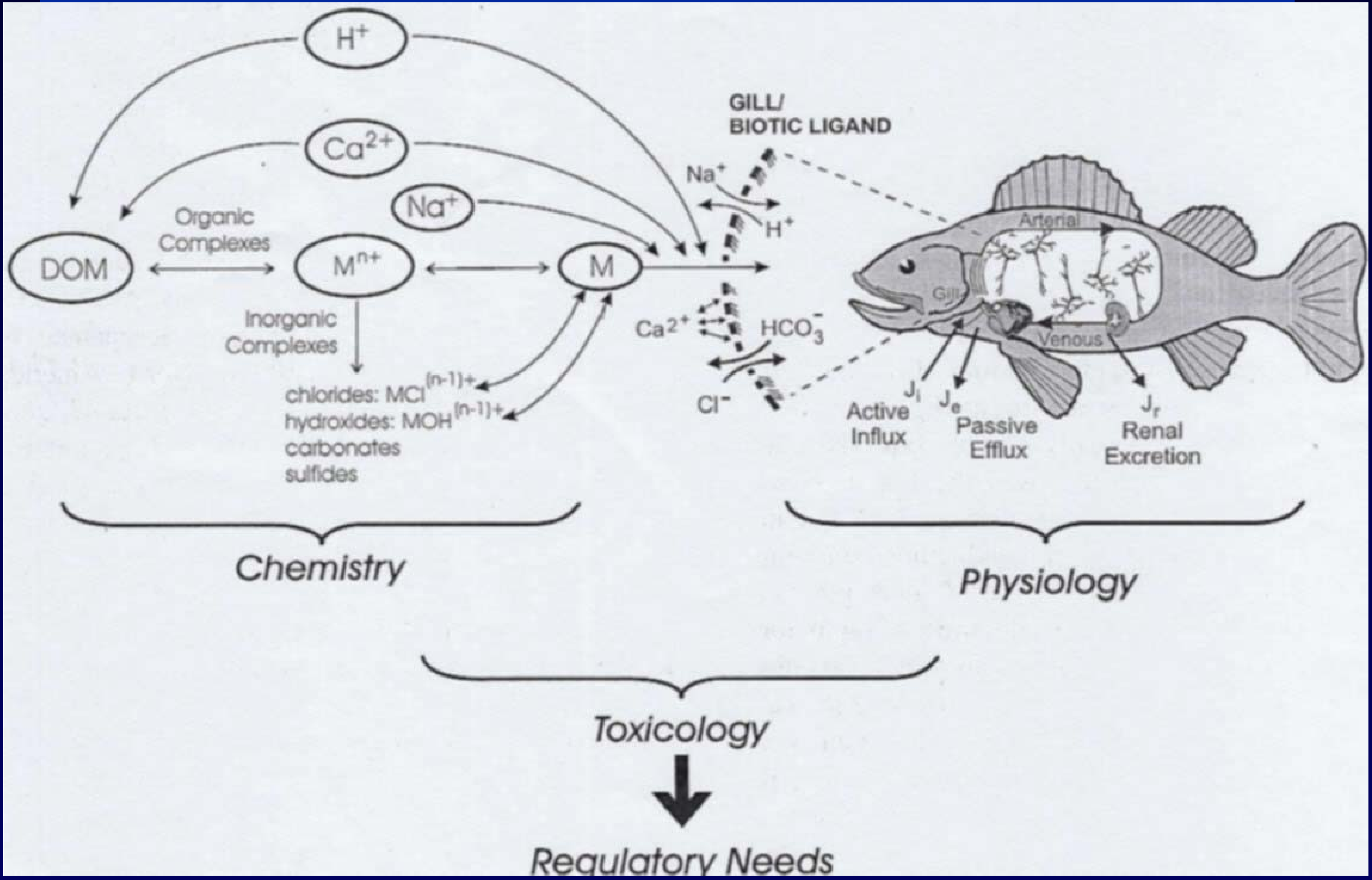


So how do we put the ecotoxicology and chemistry together?

Long-term prediction

Environmental fate models – sorption/desorption; aging

Biotic Ligand Model



Summary

- In order to generate meaningful data for hydrocarbons in ecological risk assessment of soils:
  - Biological variability is usually greater than variability of chemical measurements
  - Precision and accuracy of biological and chemical parameters must be considered
  - Bioassay assumptions must be fulfilled
- Experiments must be truly interdisciplinary (i.e., analytical chemistry, ecotoxicology, physiology, biochemistry, environmental chemistry) to obtain data useful for ecorisk

Acknowledgements

Technical assistance & brainstorming

- Nick Basta, Ohio State University, School of Natural Resources
- Karen Bradham, Jason Conder, Lisa Seals, Dean Fitzgerald, Jason Wells, Brad Knight
- Jim Huckins, USGS Columbia Research Centre
- Lynn McCarty, L.S. McCarty & Associates

Acknowledgements

Financial Assistance

- The RETEC Group (Dave Nakles)
- U.S. Environmental Protection Agency
- Oklahoma State University Environmental Institute
- SERDP – CU-1210

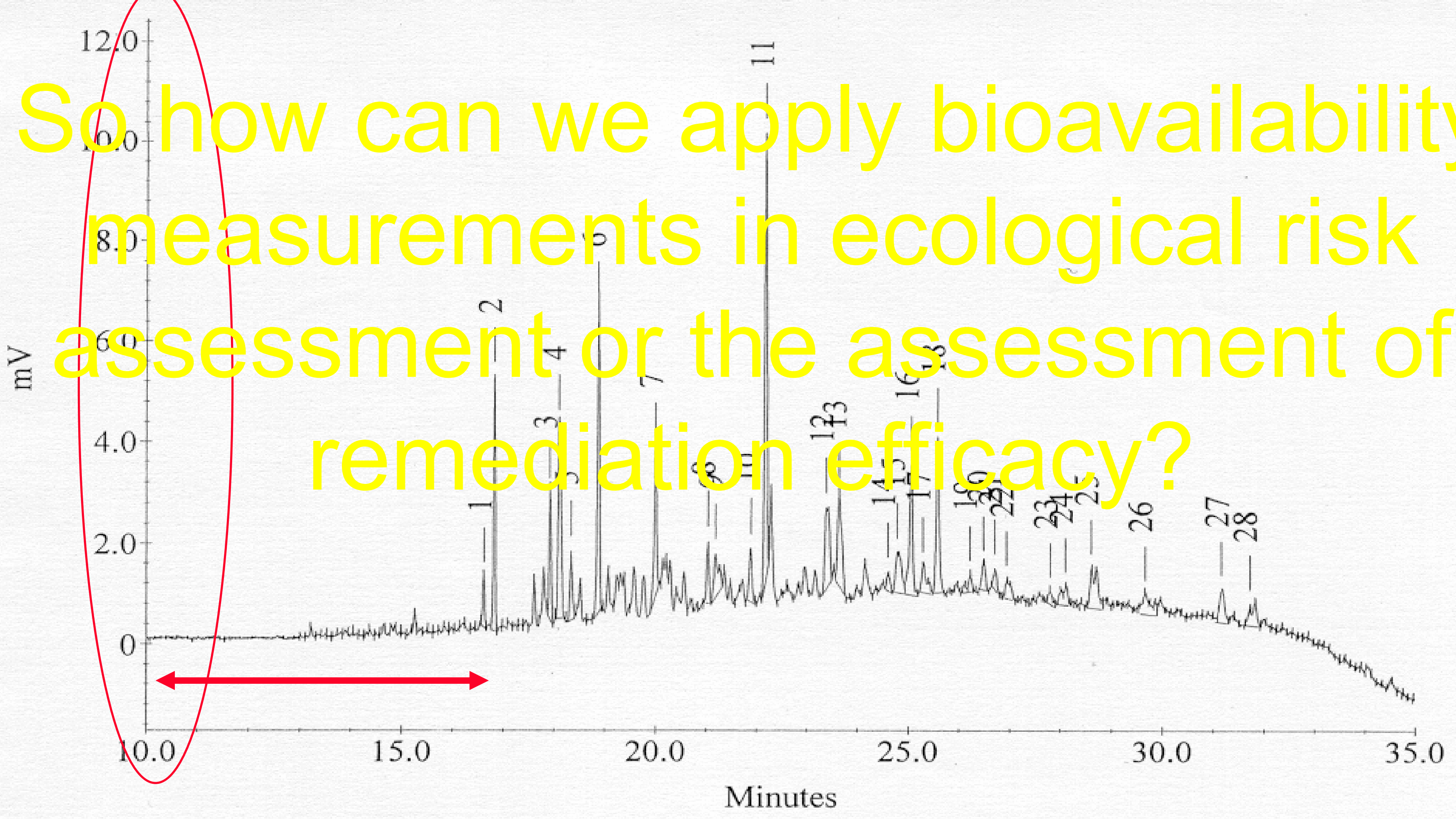
Summary

- If you're doing ecotoxicology, there is no excuse for not getting the dose and the response correct - otherwise it is just bad science
- Experiments must be truly interdisciplinary (i.e., analytical chemistry, ecotoxicology, physiology, biochemistry, environmental chemistry) to obtain data useful for ecorisk

Unanticipated effects in soil bioassays

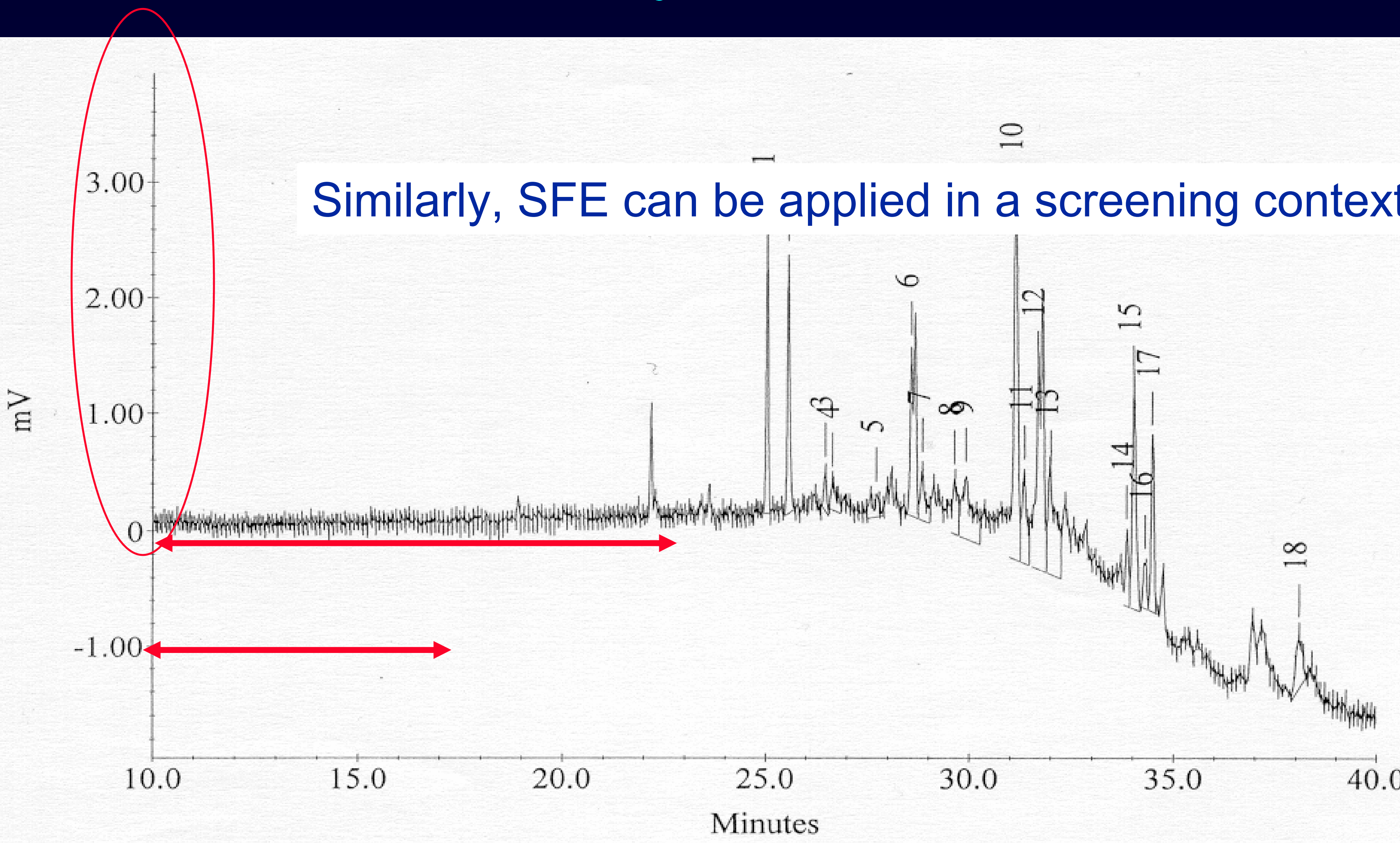
- Autocorrelated parameters
- Soil effects – texture, OC (content and type)
- Acclimation to soil, chemical
- Declining/changing dose
- Effects of organisms on soil/chemicals

Screening level assessment - SPME extraction of soil containing PAH mixture 100% mortality of earthworms



So how can we apply bioavailability measurements in ecological risk assessment or the assessment of remediation efficacy?

SPME extraction of soil containing PAH mixture 0% mortality of earthworms



Similarly, SFE can be applied in a screening context

Comparison of hazard quotients

$$\text{Hazard Quotient (HQ)} = \frac{EC_t}{TRV_t} = \frac{EC_b}{TRV_b} = \frac{\text{Residue}}{\text{CBR}}$$

Where EC = Environmental concentration (t = total; b = bioavailable)

TRV = Toxicity Reference Value (t = total; b = bioavailable)

Residue = Residue in organism

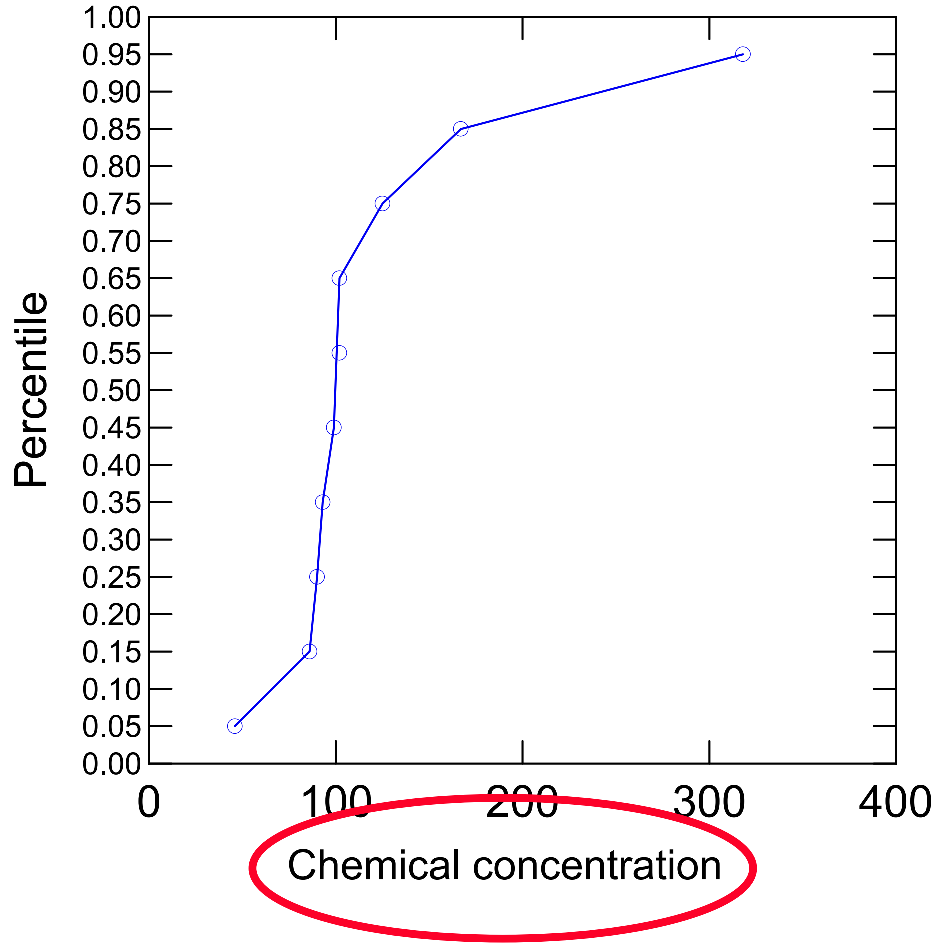
CBR = Critical body residue

How do we use these tools in environmental quality criteria?

- Pre-guideline application
  - Base guideline derivation on data from substrate with “highest bioavailability”
    - Ecological soil screening levels (EcoSSLs)
    - Data for chemicals log K_{ow}>3.5 from tests in soils with pH – 4-7, OC<2%
  - Base guideline derivation on exposure data from measures of bioavailability
    - Develop dose-response curves based upon measures such as SPME uptake

Species Sensitivity Distribution (SSD)

Probability distribution of species mean acute values (SMAVs)



How do we use these tools in environmental quality criteria?

- Post-guideline application (if exceeded)
  - Adjust guideline to site-specific conditions using various models
    - e.g., equilibrium partitioning, regression models
  - Apply tools to measure potential bioavailability and compare to established dose/response measures
  - Simply use total chemical levels!

How do we use these tools in environmental quality criteria?

- Bioavailability measurements may only make a difference when chemical levels are in the "gray" area
- Otherwise, guidelines and measurements based upon total chemical levels may suffice

Low

Apply bioavailability measures

High

Soil chemical concentration